SHORT NOTE

Eur. J. Entomol. **92**: 259–261, 1995
ISSN 1210–5759

Hormonal regulation of the *Drosophila melanogaster ng*-genes

PIER PAOLO D'AVINO, STEFANIA CRISPI and MARIA FURIA

Dipartimento di Genetica, Biologia Generale e Molecolare, Università di Napoli "Federico II",
Via Mezzocannone 8, I-80134 Napoli, Italy

Ecdysone, intermoult puff, *Broad-Complex*, gene expression

Abstract. Intermoult puffs constitute the first set of ecdysone-responsive puffs detected on salivary gland chromosomes of *D. melanogaster* third instar larave. In order to analyse the hormonal regulation of this puff set we have investigated the genetic regulatory hierarchy governing the expression of three genes mapping at the 3C polytene region, from which one of the most prominent intermoult puff originates. The results obtained reveal that the expression of the genes studied, named *ng-1*, *ng-2* and *ng-3*, is coordinate and specifically restricted to the Puffing Stage 1 (PS1). The repression of these genes appears to be related to the PS1/PS2 transition and is dependent on the activity of the ecdysone induced *BR-C* locus. While the *ng*-gene repression results to be a secondary response to ecdysone, the recent identification of functional EcREs within the *ng-1,2* coding region suggests that the induction of *ng*-genes might be part of the early ecdysone primary response at the beginning of the third larval instar.

During the third larval instar, the steroid moulting hormone ecdysone activates three temporally distinct puff sets on the *D. melanogaster* salivary gland polytene chromosome: the so-called intermoult, early and late puffs (for a review, see Ashburner & Berendes, 1978). Intermoult puffs constitute the first set of ecdysone-responsive puffs to be detected on salivary gland chromosomes of third instar larvae. These puffs are thought to be induced by a small rise in the ecdysone titre occurring at the beginning of this stage (Pongs, 1988) and repressed in response to the large increase in ecdysone concentration occurring a few hours before the metamorphosis (Ashburner et al., 1974).

The hormonal regulation of intermoult puffs is thus quite complex, and so far largely not understood. In order to investigate this aspect, our group is involved in the characterization of the *ng-1*, *ng-2*, and *ng-3* genes we have recently identified at the 3C intermoult puff region (Furia et al., 1990, 1993). The results obtained allowed us to classify these genes as a new group, with distinct properties with respect to those of the other intermoult genes so far characterized.

A precise definition of the *ng*-gene developmental expression pattern during the third larval instar has been obtained by Northern-blot analysis of single, carefully staged third-instar larvae (D'Avino et al., 1995). This type of analysis lead us to establish that the *ng*-genes are actively transcribed from the beginning of the third instar, whereas the other intermoult genes are actively transcribed only subsequently, from the beginning of the wandering stage (Andres et al., 1993).

Interestingly, the expression of *ng-1,2,3* genes appeared to be strictly restricted to the PS1 (Puffing Stage) stage, since these genes are repressed before the *E74A* subform of the *E74* early gene is induced. This finding suggests that the *ng*-gene repression might be related to the PS1/PS2 transition. This developmental transition has been suggested to be due to an increase in ecdysone titre able to activate a set of ecdysone-induced early gene promoters, including *E74A*, *E75A*, *E75B* and at least one *BR-C* promoter (Huet et al., 1993).

In order to elucidate the role of the *BR-C* locus in the genetic hierarchy regulating the expression of the *ng*-genes, we have then investigated the effects of *BR-C* null mutations on the level of accumulation of the *ng*-mRNAs. This analysis revealed a second, essential aspect differentiating *ng-1,2,3* from the other intermoult genes. In fact the *ng*-genes are actively transcribed in *BR-C* null mutants, in which the transcription of other intermoult genes is drastically reduced (Crowley et al., 1984). Conversely, the *ng-1,2,3*

gene repression, occurring at the early wandering stage, is dependent on *BR-C* functions. By using strains carrying mutations in each of the three *BR-C* lethal complementation groups, we identified the *rbp* and *2Bc* functions as those involved in *ng*-gene repression, with the *2Bc* showing the most dramatic effect. In *2Bc* animals, in fact, the *ng-1,2,3* transcripts remain abundant in both late wandering larvae and white pupae, so that in this mutant background *ng*-gene expression is not longer restricted to the PS1 stage but lasts at high level until pupariation, corresponding to the PS11 stage. This alteration in the *ng*-gene transcriptional pattern correlates well with the puffing disturbance noticed in this mutant background by Zhimulev et al. (1982) several years ago. These authors described that, as a consequence of the delay in the intermoult puff regression and of the submaximal induction of some early puffs, an unusual puff combination is detected on the chromosomes of $2Bc^{l}$ white-pupae, in which puffs characteristic of both early and middle third instar coexist, abnormally. The transcriptional pattern observed clearly represents the molecular counterpart of the puffing disturbance described for $2Bc^{l}$ mutants, with the transcription of *ng*-genes abnormally overlapping with that of *E74A*.

A small ecdysone peak has been inferred to occur immediately before wandering in *Drosophila* as well as in other holometabolous insects (for a review, see Andres et al., 1993); this peak has been connected with a variety of developmental events, such as the cessation of the feeding of the growing larvae and the initiation of wandering (Dominick & Truman, 1985). We suggest that in *D. melanogaster* this peak is responsible for the repression of *ng-1*, *ng-2* and *ng-3*. This hypothesis is also supported by previous data we have obtained with the temperature-sensitive mutant ecd^{l} mutant strain, in which the ecdysone titre is strongly reduced at the non-permissive temperature. When the ecd^{l} strain is grown at the non-permissive temperature, the mutant larvae do not start wandering, probably as a consequence of the lack of the pre-wandering ecdysone peak (Garen et al., 1977; Berreur et al., 1984). Shifting ecd^{l} larvae at the non-permissive temperature before the wandering stage leads to a strong reduction of the *Sgs* intermoult genes transcript level; in contrast, shifts made either before or after the wandering stage did not significantly affect the level of *ng*-transcripts. In both cases, however, *ng*-mRNAs are detected for a prolonged period (Furia et al., 1992).

The effects of ecd^{l} mutation on the expression of the *ng*-intermoult genes can now be better understood on the basis of the results obtained in the *BR-C* mutant background. In fact, it is reasonable to suppose that the lack of the prewandering peak occurring in the ecd^{l} strain at the non-permissive temperature causes, first of all, a failure in the *BR-C* induction. This might trigger opposite effects in the expression of intermoult genes, by simultaneously abolishing the *Sgs*-gene induction and the *ng*-gene repression.

We would like to underline here that a strong correlation exists between the timing of the 3C intermoult gene expression and that of the 3C puff occurrence. In fact, puffing at the 3C11-12 region is most prominent at PS1 and declines subsequently (Ashburner & Berendes, 1978). Considering that the *ng*-gene expression correlates well with the puff temporal profile not only in wild-type animals, but also in the *BR-C* mutant larvae, it is reasonable to suppose that transcription of the *ng-1,2,3* genes might play an active role in triggering the 3C puffing. Very recently, we have investigated the possibility that *ng-1,2,3* were primary-response genes by searching for *ng*-regulatory elements behaving as high affinity ecdysone receptor binding sites. The search for such elements, performed by means of the DNA-blotting assay (Cherbas et al., 1991), has lead to the identification and the characterization of two EcREs located within the *ng*-1 and *ng*-2 coding regions (D'Avino et al., in prep.). Surprisingly, these elements turned out to be composed by directly repeated half-sites, instead to be palindromic as all the other previously described EcREs, indicating that the repertoire of EcREs mediating the ecdysone response in vivo is much larger and more degenerate than previously suspected.

A further interesting property displayed by the *ng*-EcREs is their ability to bind not only the EcR/USP heterodimer, but also USP alone (D'Avino et al., in prep.). The fact that both EcR/USP and USP can bind to the same response element clearly implies that they may compete for DNA binding, a finding that may be relevant for the transcriptional regulation of *ng-1,2,3* as well as for other ecdysone-regulated genes.

In conclusion, all the data discussed above suggest that the expression of *ng-1*, *ng-2* and *ng-3* is part of the ecdysone primary response to the ecdysone at the beginning of the third larval instar.

ACKNOWLEDGEMENTS. This work was supported by grants of the CNR Target Project on Biotechnology and Bioinstrumentation and MURST 40% and 60% to M.F.

References

ANDRES A.J., FLETCHER J.C., KARIM F.D. & THUMMEL C.S. 1993: Molecular analysis of the initiation of insect metamorphosis: a comparative study of Drosophila ecdysteroid-regulated transcription. *Dev. Biol.* **160**: 388–404.

ASHBURNER M. & BERENDES H.D. 1978: Puffing of polytene chromosomes. In Ashburner M. & Wright T.R.F. (eds): *The Genetics and Biology of Drosophila. Vol. 2b.* Academic Press Inc, London, England, pp. 319–395.

ASHBURNER M., CHIARA C., MELTZER P. & RICHARDS G. 1974: On the temporal control of puffing activity in polytene chromosomes. *Cold Spring Harbor Symp. Quant. Biol.* **38**: 655–662.

BERREUR F., FORCHERON F., MORINIERE M., BERREUR-BONNENFANT J., BELINSKI-DEUTSCH S., BUSSON D. & LAMOUR-AUDIT C. 1984: Ecdysteroids during the third larval instar in l(3)ecd-1st, a temperature-sensitive mutant of Drosophila melanogaster. *Gen. Comp. Endocrinol.* **54**: 76–84.

CHERBAS L., LEE K. & CHERBAS P. 1991: Identification of ecdysone response elements by analysis of the Eip28/29 gene. *Genes Dev.* **5**: 120–131.

CROWLEY T.E., MATHERS P.H. & MEYEROWITZ E.M. 1984: A trans-acting regulatory product necessary for expression of the Drosophila melanogaster 68C glue gene cluster. *Cell* **39**: 149–156.

D'AVINO P.P., CRISPI S., POLITO L.C. & FURIA M. 1995: The role of the Broad Complex locus on the expression of genes located at the ecdysone-regulated 3C puff of Drosophila melanogaster. *Mechs. Dev.* **49** (in press).

DOMINICK O.S. & TRUMAN J.W. 1985: The physiology of wandering behavior in Manduca sexta II. The endocrine control of wandering behavior. *J. Exp. Biol.* **117**: 45–68.

FURIA M., DIGILIO F.A., ARTIACO D., GIORDANO E. & POLITO L.C. 1990: A new gene nested within the dunce genetic unit of Drosophila melanogaster. *Nucleic Acids Res.* **18**: 5837–5841.

FURIA M., D'AVINO P.P., DIGILIO F.A., CRISPI S., GIORDANO E. & POLITO L.C. 1992: Effect of ecd^{1} mutation on the expression of genes mapped at the Drosophila melanogaster 3C11-12 intermoult puff. *Genet. Res. Camb.* **59**: 19–26.

FURIA M., D'AVINO P.P., CRISPI S., ARTIACO D. & POLITO L.C. 1993: Dense cluster of genes is located at the ecdysone-regulated 3C puff of Drosophila melanogaster. *J. Molec. Biol.* **231**: 531–538.

GAREN A., KAUVER L. & LEPESANT J.A. 1977: Roles of ecdysone in Drosophila development. *Proc. Natl. Acad. Sci. USA* **74**: 5099–5103.

HUET F., RUIZ C. & RICHARDS G. 1993: Puffs and PCR: the in vivo dynamics of early gene expression during ecdysone responses in Drosophila. *Development* **118**: 613–627.

PONGS O. 1988: Ecdysteroid-regulated gene expression in Drosophila melanogaster. *Eur. J. Biochem.* **175**: 199–204.

ZHIMULEV I.F., VLASSOVA I.E. & BELYAEVA E.S. 1982: Cytogenetic analysis of the 2B3-4-2B11 region of the X-chromosome of Drosophila melanogaster. III. Puffing disturbance in salivary gland chromosomes of homozygotes for mutation l(1)pp1t10. *Chromosoma* **85**: 659–672.